THE RUMEN MICROBIAL ECOSYSTEM DURING THE TRANSITION PERIOD IN DAIRY COWS

BY

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THESIS

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ABSTRACT

Seven rumen cannulated Holstein cows were used from 3 weeks prepartum to 4 weeks postpartum to determine the relative abundance of 7 different species of ruminal microorganisms. The prepartum diet was based on corn silage. In the postpartum, diet included ground corn, grain by-products, and alfalfa haylage. Ruminal digesta were collected at five times: -14, -7, 10, 20, and 28 days around parturition. Total DNA from ruminal digesta was isolated and real-time quantitative PCR was used to determine the relative abundance of bacterial species. Eubacterium ruminantium and Selenomonas ruminantium were not affected by time ($P>0.05$). Megasphaera elsdenii and Prevotella bryantii increased significantly postpartum ($P<0.001$). Conversely, Butyrivibrio proteoclasticus decreased gradually from -14 through 28 days ($P<0.001$). Fibrobacter succinogenes was affected by time being lowest at day 10 ($P=0.02$) while Anaerovibrio lipolytica recorded the lowest abundance at -7 d followed by an increase by 20 days postpartum ($P<0.001$). Overall, these results indicate that changes in diet after parturition affect the abundance of ruminal bacteria, particularly M. elsdenii (a lactate-utilizing bacteria) and P. bryantii (a starch-degrading bacteria) which increased markedly after parturition likely as a consequence of a higher concentrate intake. In terms of milk production, there was a relative decrease in the body weight of dairy cows.The milk yield increased considerably up to the fourth week, while the percentage of fat was reduced considerably.

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LIST OF ABBREVIATIONS

AA	Amino Acids
BCS	Body Condition Score
BR	basal-Ration
BW	Body Weight
CHO	Carbohydrate
CT	Threshold Cycle
DF	Disodium Fumarate
DFM	Direct-Fed Microbial
DM	Dry Matter
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
EB	Extraction Buffer
EDTA	Ethylenediaminetetraacetic Acid
H	Holstein
K	Ketosis
NEB	Negative Energy Base
NFC	Non-Fiber Carbohydrate
PCR	Polymerase Chain Reaction
RL	Reticulo-Lumen
SARA	Sub-acute Ruminal Acidosis
SDS	Sodium Dodecyl Sulfate
TC	Transition Cows
TE	Tris-EDTA Buffer
TMR	Total Mixed Ration
TRFLP	Terminal Restriction Fragment Length Polymorphism
TS	Tea-Saponins
VFA	Volatile Fatty Acid

INTRODUCTION

The transition phase is the most crucial period in a dairy cow's health and productivity. However, dairy cows experience a lot of hormonal, metabolic, as well as dietary changes during the transition period. As a result, this phase requires a lot of preparation and care with regard to managing dairy cows (Grummer, 1995). This is attributed to the fact that, most dairy cattle acquire diseases during this period. This is often as a result of increased periods of Negative Energy Base (NEB). In trying to reduce NEB, it is crucial to start feeding dairy cows with energy dense diets three weeks prior to delivery (Ishler, 1996). However, this process may result in the overconsumption of energy and enhance the risk of metabolic ailment postpartum. Prepartum, as well as postpartum intakes suggest that enhanced nutrient density of prepartum feeds could enhance Dry Matter Intake (DMI) and reduce NEB postpartum. As a result, the health and productivity of dairy cattle in the transition period is directly affected on by nutrition, performance during the initial days of lactation. The transition period marks a crucial phase from late pregnancy and the initial lactation days (Benabucci, 2002). Since the transition phase is the most crucial period in a dairy cow's health and productivity, it is crucial to consider all aspects in relation to a cow's dietary needs to eliminate NEB (Grummer, 1995). This will allow the cow to stay healthy in a period of many hormonal changes. According to Erdman (1988), these precautionary preparations will take into consideration the changes that occur to ruminal bacteria populations that are critical in maintaining the rumen of the dairy cow healthy. Without a healthy and functioning rumen, the dairy is bound to have a lot of health problems which will eventually impact

the cow's milk production. When the cow's productivity goes down, then her profitability also decreases (Erdman & Moreland, 1989; Anderson, Sehested, & Ingvartsen, 1999).

CHAPTER I. LITERATURE REVIEW

The peripartal period

Customarily, the management of cows that are not lactating is by neglect (Roche, 2000). Substandard care and feeds can decrease the quantities of milk that the cows give, and increase the prevalence of health-related disorders in them. Dry-periods are commonly typified as durations when the cows have minimum nutritional needs. Such characterization presents an erroneous notion regarding the period's significance in relation to the cows' prospective production and health. In a study by Roche, Dalley, Moate, Grainger, O'Mara and Rath (2000) it is held that a cow's transition-period is the time interval between 27 days prior to calving and 27 days after the calving date. During the period the cow's priority with regard to nutrients adjust to condition-score-loss from condition-score-gain. Nutrition-related requirements of the cow go up with growing fetal demands along with increasing activity in its mammary glands (Roche, Dalley, Moate, Grainger, O'Mara & Rath, 2000). Increasing amounts of nutrients are necessary for supporting the continuing maintenance and growth of the glands, fetus, uterus as well as placenta (Roche, 2000).

The productivity of cows in the period is directly impacted on by nutrition, performance during early days of lactation, and any extant metabolism-related disorders. Van Saun (2009) advises that optimizing the care given to the cows during the period occasions increased and sustained productivity and outcomes regarding breeding-pens and health. During the 27-day prepartum period the cows' DMI (Dry Matter Intake) may go down by almost a third (Overton & Boomer, 2009). If a cow goes off-feeding before it calves it becomes highly susceptible to developing metabolism-related disorders. Such disorders stunt their performance in the course of the early days of lactation, and

adversely impacts on their reproduction and production capacities when lactating. Overton and Boomer (2009) advise that before calving the cows should be relocated into pens that have not been utilized before. This enables farmers to make out the ones that will calve and the ones that will become off-feed. Notably, cows that are moved to new-animal pens may consume less feed when the transition is underway. Lengthy times can be expended in reconfiguring the hierarchies of the animals. During the transition, the cows' rumens undergo significant changes as the cows transition from feeds that are high in forage but low in energy to feeds that are high in energy but low in forage. The changes impact, directly, on populations of microbials in the rumens. Consequently, it is critical that the rumens are readied for those changes in rations (Overton & Boomer, 2009; van Saun, 2009).

According to Formigoni and Trevisi (2003), the duration and intensity of deficit of energy in transition cows (TC) are both inversely linked to their indices of production. If the deficit is serious it suppresses gonadotrophins' secretion, levels of insulin in the cows' plasma, and progesterone's secretion. Cows with suppressed secretion of progesterone have high probabilities of fetal abortions. Diet impacts on their fertility in a number of ways. Too much proteins, which are degradable, in the rumen occasion balances of energy that are negative and that adversely impact on the cows' reproductive processes (Formigoni & Trevisi, 2003). An extensive search for relevant researches lends support to the conclusion drawn by Chen, Penner, Li, Oba and Guan (2011), that the present appreciation of communities of epimural-bacteria in TC and other animals is limited. The bacteria are associated with the epithelial-tissues of rumens (Chen, Penner, Li, Oba & Guan, 2011).

The study by Chen, Penner, Li, Oba and Guan (2011) provides ample insights into whether the influences that diet has on TC', especially in relation to epimural-bacteria's diversity in their rumen. They characterize the diversity and approximate the entire population of the bacteria (number of 16S-rRNA-gene copies). During transition of diet, which is rapid, the diversity changes in (Rapid-Grain Adaptation) RGA heifers but remains unchanged in the control ones (Chen, Penner, Li, Oba & Guan, 2011). Overton and Waldron (2004) determine that dairy TC undergo marked metabolism-related adaptations in fatty acid, salt, and glucose metabolism in supporting lactation as well as avoiding related dysfunctions. During the transition, the management of the TC' nutrition is geared towards supporting the adaptations. Overton and Waldron (2004) indicate that considerable research has been executed regarding the adaptations and the strategies that should adopted in the management. There is growing support for two-group strategies for managing the nutrition in the case of dry-cows. This is to reduce nutrients' overfeeding in early days of the dry duration and increase the supply of nutrients to support metabolism-related adjustments to lactation in the latter days of the duration (Overton & Waldron, 2004).

In the prepartum phase increased energy supplies via carbohydrates give rise to enhanced TC' metabolism as well as performance. Even then, recent studies show that the make up of the carbohydrate is of lower, almost insignificant importance. Feeding TC dietary sources of fat, or lessening the energy that they expend through feeding them particular fatty acids to lessen the output of fat in milk, in the early days of lactation, results in nil changes in the release of the adipose layer fatty acids that are not esterified. Recent researches, that are reviewed by Overton and Waldron (2004) show that there are

probable physiological connections between the presence of derived metabolism-related disorders in TC and incidence of primary diseases that are infectious among them. Jouany (2006) submits that the deficits in intake of energy among TC means that the ones with high productivity have need of rations that are dense in energy. TC' rumen-based microbes ferment diets that are rich in starch, resulting in high VFAs' (Volatile Fatty Acid) generation, lower pH, in addition to lactic-acid accumulation. The acid speeds up the pH's decline. Furthermore, it commonly results in rumen-acidosis in TC (Jouany, 2006).

The acidosis causes the malfunctioning of TC' rumens, impacts on rumen-based microbes, and makes digestion ineffective thus reducing the TC' intake of feed and heightening the deficit. The management of diet is the principal way of limiting the acidosis' risk. TC' ought to be incrementally fed on grain-ingredients during the pre-calving transition interval. During the interval TC should be fed on adequate fiber for purposes of stimulating salivation along with rumination. Starch, whose degradability is low, should be privileged. Some chemicals can be added to feed to stem the occurrence of acidosis. Such chemicals include pH buffers to control pH in the TC' rumens. Lactic acid's synthesis can be inhibited by metabolic-hydrogen whose mobilization is done via particular pathways of metabolism like propionogenesis**.** The mobilization can be facilitated by the addition of precursors of propionate like fumarate, aspartate as well as malate. Notably, consumers of TC' milk are normally against the usage of the precursors, which are economically unviable. Other additives that are used in preventing the acidosis include probiotics, whose action pathways are presently not wholly understood. Various antibiotics that are used in managing acidosis in TC and other animals may potentially

inhibit bacteria that are gram positive. In 2006, the EU banned the antibiotics' application as additives in feeds made for animals, including TC (Jouany, 2006).

Rumen microbes

To make certain TC attain their gene-determined potential for generation of milk in addition to being healthy, their rumens ought to be health. Dysfunctional rumens impair the digestion of TC feed, with the cows becoming quite prone to varied metabolism-related diseases (Chen, Penner, Li, Oba & Guan, 2011; Yang, Beauchemin & Rode, 2001; Yang, Beauchemin & Rode, 2002). Populations of ruminal microbes compete with the TC for nutrients. They impact on the ecosystem of the rumen, and eventually on the availability of the nutrients that TC require for the purpose of production. The microbes in the rumen include fungi, protozoa along with bacteria. Largely, the protozoa are ciliated and the fungi are anaerobic (Yang, Beauchemin & Rode, 2002). Ruminal populations of the microbes are markedly dynamic. Characteristically, they change rapidly with diet-related factors (Chen, Penner, Li, Oba & Guan, 2011; Yang, Beauchemin & Rode, 2001).

Nagaraja and Titgemeyer (2007) opine that the degree of intricacy of the relations between varied microbes or their groups found in rumens is so high that to date, several microorganisms and pathways that are involved remain unknown. Microbiologists are now increasingly interested in developing ways of controlling varied metabolism-related processes that happen within rumens. Given the continued negative perceptions that the public has against the utilization of various chemical agents (antimicrobials) in the control of microbes, growing efforts are being directed towards formulation of substitute additives like organic-acids, enzymes, probiotics, and others (Jouany, 2006; Nagaraja & Titgemeyer, 2007).

The formulation and production of substitutes to the range of antibiotics in promoting growth in TC agriculture is now a priority. Even then, concerns about milk safety in recent years have continually directed researchers' attention towards microbes like *Escherichia coli* (O157:H7), which is associated with numerous food-related illnesses. There are continuing efforts to lessen TC microbe shedding (Nagaraja & Titgemeyer, 2007; Tricarico, Abney, Galyean, Rivera, Hanson, McLeod & Harmon, 2007; Vasconcelos, Elam, Brashears & Galyean, 2008).

In a study by Park, Titgemeyer, Cochran, DeFrain, Ferdinand, Wallace, Nagaraja, Johnson and Shirley (2010) use Holstein TC that are pregnant, ruminally-fistulated as well as multiparous in delineating adaptations of microbes between lactations. The diets that are offered to the TC comprise of close up as well as far off diets, a diet used in the latter days of lactation and that contains 20% DM (dry matter) in form of feed of wet-corn type of gluten, and silage from hay-corns of alfafa, which is based on a diet made for the early days of lactation. Samples of microbes are collected on the 72^{nd}, 51^{st}, 23^{rd}, and 9^{th} days before calving, and the 6^{th}, 20^{th}, 34^{th}, 48^{th}, 62^{nd}, 76^{th}, and 90^{th} days postpartum. Ruminal-samples are analyzed for protozoa, which are ciliated, and fungi as well as bacterial counts. It is established that transiting from a diet that is rich in foliage to one that is rich in concentrates has lower impact on counts of bacteria than it has on counts of protozoa as well as fungi. Transiting from diets that are rich in concentrates to ones that are rich in forage grows the counts of the protozoa as well as fungi. The counts reduce when transiting from diets that are rich in forage to ones that are rich in concentrates. There is an increase in the counts of bacteria and protozoa in the early days of lactation (Park et al., 2010).

The counts go down as TC move towards peak DMI. Changes in diet when lactation is starting actualize ruminal fungi's near disappearance. Generally, populations of microbes in dairy cattle's rumens are impacted on by diet modifications as well as intake. Protozoan along with fungal populations are markedly impacted on by dietary changes, while intake changes impact on protozoan, bacterial in addition to fungal populations (Park et al., 2010). Wang, Li, Zhao, Hu, Chen, Liu, Liu and Wang (2012) submit that the species and sizes of populations of microbes heavily influences processes such as gluconeogenesis. Gluconeogenesis is the processes through which glucose is generated by propionate. They examine the composition of microbiome in rumens of TC, cows that have ketosis (K) as well as non-perinatal ones. The examination is executed via "terminal restriction fragment length polymorphism (TRFLP)" 16S-rRNA-gene analysis and PCR that is quantitative (Wang, Li, Zhao, Hu, Chen, Liu, Liu & Wang, 2012).

The analysis establishes that there is reduction of *Veillonellaceae* microbe quantities, growth of *Streptococcaceae* microbe populations in the TC as well as in the K groups. *Lactobacillaceae* microbe populations grow following calving. Data got from the PCR shows decimation of populations of microbes that are the principal producers of propionate; *Selenomonas ruminantium* along with *Megasphaera elsdenii*. The ruminal population of the bacteria that are the principal producers of lactate, *Streptococcus bovis* grow in TC and in the K groups. Following calving, the *Lactobacillaceae* microbe populations grow. The concentrations of glucose and VFA decrease while that of lactic acid goes up in the samples obtained from the K groups and TC. The researchers demonstrate a close connection between the concentration of VFA and the size of

ruminal populations of *S. ruminantium* along with *M. elsdenii* (Wang, Li, Zhao, Hu, Chen, Liu, Liu & Wang, 2012).

Chen, Penner, Li, Oba and Guan (2011), examining the influences that diet has on ruminal epimural-bacteria population diversity in RGA-heifers, detect bacteria of *lachnospiraceae*, *treponema* along with *ruminobacter* species in cases where the heifers feed on diets containing 0.08 and 0.25 hay. The bacteria's presence is amply attributed to adaptations to diets that are high on grains. The population is positively linked to molar ratios of isovalerate, acetate along with isobutyrate. It can be inferred from the positive link between the ratios and the population that isovalerate, acetate along with isobutyrate affect the ruminal metabolism of VFA (Chen, Penner, Li, Oba & Guan, 2011). Nocek and Kautz (2006) examine the supplementation effects of "direct-fed microbial agents (DFM)" on TC (p.260). They establish that if the supplementation of DFMs is broadly targeted, it improves the digestion of DM-forage in the rumen. In the early days of lactation cows that receive DFM as supplement have increased milk production. They take in higher quantities of DM during the transition time. The proportion of fat in their milk is lower compared to milk from cows that are not supplemented (Nocek & Kautz, 2006).

Ruminal bacteriophages have over a hundred varied morphologies and are effortlessly viewed under microscopes (Edwards, Huws, Kim, Lee, Kins ton-Smith & Scollan, 2008). They are either of temperate or lytic forms. Temperate bacteriophages are the most common in TC' rumens. The lytic ones considerably influence the forms as well as numbers of ruminal bacteria. There are possibilities that they make contributions to microbe populations' homeostasis (Calsamiglia, Cardazo, Ferret & Bach, 2008).

Bacteriophages are highly particular on their hosts. Their treatment is evidently easy owing to their units that self-replicate. Bacteria may develop resistance against the bacteriophages. Therapies that are built on bacteriophage units, for eliminating particular microbes such as *S. bovis*, *E. coli* (O157:H7) and related pathogens, or bacteria that produce hyper-ammonia present marked benefits (Busquet, Calsamigli a, Ferret & Kamel, 2006). Wide-ranging searches for studies that relate to ruminal bacteriophages show that the bacteriophages' dynamics of population, general functional dynamics and significance, and biology have not been conclusively determined.

Given that varied microbes share their habitats, competing for available substrates, they interact in both intricate and straightforward ways, which are adverse or beneficial to their hosts and to themselves. Research has established that ruminal protozoa and bacteria horizontally exchange genetic matter. The transfer of the matter may present the receiving microbes with new functions. Given that ciliates are capable of engulfing as well as digesting bacteria, the ciliates may absorb and incorporate bacterial DNA into their own genomes according to Ricard et al. (2006).

For instance, *Polyplastron multivesiculatum* produces xylanase that is closely comparable to xylanases produced by bacteria that are gram-positive (Devillard, Newbold, Scott, Forano, Wallace, Jouany & Flint, 1999). Ruminal microbes' sequences of genomes can give detailed information regarding their potential relating to metabolism according to Suen et al. (2011). Information extracted from bacteria's genomics, especially if the bacteria is fibrolytic, gives ample insights in how fibers are digested within animals', including TC', rumens. There are possibilities that the information can ease the making out of enzymes that can be utilized in commercial applications such as

being applied as additives to foods or used in generation of bio-fuels as established by Hess et al. (2011). *Methanobrevibacter ruminantium* has had its genomes sequenced as well as analyzed in two studies. The microbe is a principal methanogen in rumens. In both studies its methanogen-particular genes, which ensure the coding of essential enzymes when methane is produced, have been successfully made out. In mitigation the genes may be effectively targeted (Attwood, Altermann, Kelly, Leahy, Zhang & Morrison, 2011; Leahy, et al. 2010).

The microbe has numerous genes, which, as proteins, are capable of encoding in relation to surface-adhesion. The genes might play considerable roles in the mediation of close relations with protozoa or even bacteria that generate hydrogen within rumens. Such proteins may serve antigen roles in vaccines made for inducing methanogen-inhibition in rumens (Attwood, Altermann, Kelly, Leahy, Zhang & Morrison, 2011). This means that all efforts aimed at linking the microbes in rumens to the physiologies of their hosts or parameters of generation ought to be sensitive to variations in the structures of the microbe communities at the level of strains as well as species. Wide-ranging searches for past researches relating to the variations show that limited studies have been conducted to establish the connections between the structures and the phenotypical characteristics of the microbes' hosts.

Ruminal fermentation

Generally, the rumen is viewed as an open chamber in which fermentation happens, and which hosts anaerobic microorganisms. The microorganisms anaerobically break down complex feed components and give off products such as acids as well as microbe cell-masses that hosts consume. The products of the ruminal process of fermentation, especially VFA, are absorbed into the hosts' blood (Nocek, Kautz, Leedle

& Allman, 2002). The cells of the microbes that enter the duodenum and abomasum help in meeting the hosts' protein along with energy needs. The ruminal fermentation environment, built on interactions of the microbes and the hosts, function like continual and effectual systems of cultures that have the chemical as well as physical conditions that support the activities and growth of microbes. Ideally, the environment should be continually supplied with varied essential substrates, and cleared of undigested materials and byproducts of fermentation (Lettat, Nozière, Silberberg, Morgavi, Berger & Martin, 2012; Bannink, Gerrits, France & Dijkstra, 2012; Nocek, Kautz, Leedle & Allman, 2002).

Bannink, Gerrits, France and Dijkstra (2012) establish that during the early days of lactation the epithelia of rumens change transiently owing to variations in supplemental feeding on concentrates. They conclude that rapid increase of the intake of concentrates does not adversely impact on fermentation in the rumen, DMI, and performance of TC. The researchers contend that this, and other conclusions that they arrive at are refuted or concurred with by other studies in extant literature. The epithelia, especially in TC in the peri-parturient phase, react in manners that are coordinated to hasty changes in diet. The coordination is essential in maintaining optimal fermentation activity and other functions of the rumen (Bannink, Gerrits, France & Dijkstra, 2012). Fermentations that occur in the rumens compromise the efficacy of probiotics that are used in managing lactic acidosis. Even then the efficacy is considerable in unstable ecosystems within rumens (Lettat, Nozière, Silberberg, Morgavi, Berger & Martin, 2012).

Essentially, the microbe-related fermentation that happens in rumens is modifiable through varied interventions at the levels of the microbes, host TC or other animal, and feed. At the animal and feed levels, the interventions have indirect impacts on the fermentation. They are aimed at altering the host animals' physiologies or feedstuffs. At the other level, the impacts are considerably direct. They alter the patterns that typify the fermentation via microbes' actions that are triggered by particular dietary additives (Krehbiel, Rust, Zhang & Gilliland, 2003; Nocek, Kautz, Leedle & Allman, 2002). Wang, Ye and Liu (2012) investigate whether tea-saponins (TS) affect fermentation in the rumens of sheep. The results give ample insights regarding the effects that the TS have on fermentation in TC. TS are generated from tea shrubs' roots, leaves, and seeds. They are exclusively pentacyclic-triterpenes, and have enduring effects although their impacts on populations of methanogens are limited. TS restrain the activity of methanogens, directly, by depressing the population of protozoa, which are ciliated. Additionally, TS decimate populations of ruminal fungi in *in-vitro* type of fermentations. The decimation happens in rumen fluid-containing media (Wang, Ye & Liu, 2012).

The TS have quite minimal impacts on patterns of fermentation in rumens and on the digestion of nutrients. In goats, TS enhances their daily gain of mass and diet efficiency (Wang, Ye & Liu, 2012). Zhou, McSweeney, Wang and Liu (2012), determining the impacts that DF (disodium fumarate) has on populations of microbes and characteristics of fermentation in the rumen, establish that some bacteria that are cellulolytic or proteolytic respond in a positive manner to DF additions. These include *Clostridium* sp, *F. succinogenes, Butyrivibrio fibrisolvens, Ruminococcus albus, P. ruminicola,* and others (Zhou, McSweeney, Wang & Liu, 2012, p.815).

Rumen Tissues

The morphologies and statuses of the epithelium of rumens play critical roles in the capacity of TC' to deal with changed nutritional regimens. The papillae of TC' rumens hastily respond to elevated VFA concentration. VFA is generated by ruminal microorganisms when they are responding to raised concentration of Non-Fiber Cabohydrate (NFC) in diets, in relation to characteristic rations that are rich in forage in the latter days of pregnancy. Consequently, the epithelium should be primed for enhanced absorptive abilities and reduction of acidosis risks when transition is underway. The accumulation of VFA in the rumen causes the acidosis as the epithelia cannot actualize adequate absorption (Penner, Aschenbach, Gabel, Rackwitz & Oba, 2009). When the elimination of ruminal VFA is raised, in responding to their mounting concentration in the rumen, the priming becomes apparent according to Aschenbach, Bilk, Tadesse, Stumpff and Gäbel (2009), McLeod and Baldwin (2000), and Argov-Argaman, Eshel, Moallem, Lehrer, Uni and Arieli (2012).

Bannink, France, Lopez, Gerrits, Kebreab, Tamminga and Dijkstra (2008) critique varied mechanisms related to reticulo-ruminal VFA production, VFA absorption across walls of reticulo-lumen (RL) as well as intra-epithelial VFA metabolism in the RL epithelium. They develop a model, which is mechanistic, to represent every one of the three aspects discretely. Given that RL tissues may markedly adjust to varying dietary conditions or circumstances, this response, which is adaptive in nature, is incorporated into the constructed model. Notably, the model is assistive in appraisal of the connections between yields of VFA and how the tissues that make up the wall of the rumen develop, VFA transport, the intensity of intra-epithelial VFA metabolism in the RL epithelium as

well as the impacts ruminants with VFA (Bannink, France, Lopez, Gerrits, Kebreab, Tamminga & Dijkstra, 2008).

The model provides ample insights into the role of the tissues in the metabolism along with absorption of molecules of VFA. Further, it appears assistive in making distinctions between elements, which require particular consideration in the examination of VFA's net-portal emergence rates and in evaluation of hypotheses relating to the relations between intra-epithelial VFA formation, metabolism, and absorption in different experimental settings (Bannink, France, Lopez, Gerrits, Kebreab, Tamminga & Dijkstra, 2008).

An extensive search for articles on the subject of the tissues of TC rumens reveals that there has been scanty research on the same. The connections between compositions of diets and the morphologies of the tissues or walls of rumens are largely inconclusive (Argov-Argaman, Eshel, Moallem, Lehrer, Uni & Arieli, 2012). It has been shown that increasing the concentration of ruminal VFA in goats through daily injections with 0.5 kilograms of butyrate, for forty two days, enlarges the surface-area of their papillae. Notably, the papillae's length as well as breadth remains constant. The papillae of heifers changes when their feeding changes from diets that are rich in fiber to diets that are rich in energy (Argov-Argaman, Eshel, Moallem, Lehrer, Uni & Arieli, 2012; Shen et al., 2004).

Goselink, van Baal, Widjaja, Dekker, Zom, de Veth and van Vuuren (2012), report that choline that is protected by the lumen lessens the accumulation of hepatic-triacylglycerol in the case of TC in the peri-parturient phase, in the early days of lactation. Reynolds, Dürst, Lupoli, Humphries and Beever (2004) determine the impacts

of supplemental along with transition proteins that are protected by the lumen or barley on the mass of visceral-tissues in TC. They also determine the impacts of barley as well transition on the volume of tissues that make up the lumen, and lumen's turnover of liquids (Reynolds, Dürst, Lupoli, Humphries & Beever, 2004).

Each cow is separately fed on grass gestation diet that is based on silage to suffice its protein along with energy needs for stasis of body mass commencing six weeks prior to the anticipated calving. Each cow is separately fed on corn lactation diet, *ad libitum*, following calving. For the study of visceral-masses, 36 TC are arbitrarily apportioned one out of the possible three nutritional treatments: 0.8 kilograms of barley-meal DM and basal-ration (BR) every day, or BR everyday, or 0.75 kilograms of soybean-based protein DM that is protected by the rumen, everyday (Reynolds, Dürst, Lupoli, Humphries & Beever, 2004).

The slaughtering of the cows is carried out at seven and 21 days prior to the anticipated date of calving or at 22 and ten days after the date when calving occurs. Characteristics of the papillae in the lumens, and the masses are determined. The study establishes that the masses are minimally impacted on by diets. On the 22nd day after calving and prior to increment of the intake of DM the masses of large and small intestines, livers, and reticulo-lumen are greater. No changes in the masses are seen at the 10th day after calving and prior to increment of the intake of DM. Masses of the papillae go up at the 10th day possibly owing to growing concentrates. Following calving, there is a decrease of mesenteric type of fats, mirroring mobilization of body-fats (Reynolds, Dürst, Lupoli, Humphries & Beever, 2004).

Ten TC, whose rumens have been cannulated, are given gestation BR on its own or accompanied by 0.88 kilograms of DM of meal made from barley. The rates of dilution of the liquids and volumes of rumens are determined at the 8th and 17th day prior to calving as well as on the 10th, 20th, and 31st day after calving. Feeding the TC on barley shows no significant consequences. Following calving, the rates and volumes of ruminal DM go up although the volumes of the liquids are unchanged. Changes that typify masses of TC' livers and gastro-intestines are occasioned by DM-intake changes along with changes in the supply of nutrients as opposed to the commencement of lactation in isolation (Reynolds, Dürst, Lupoli, Humphries & Beever, 2004).

According to Anderson, Sehested and Ingvartsen (1999) raising the intake of carbohydrate (CHO) by heifers, four weeks prior to parturition, neither changes ruminal papillae histological parameters neither their macroscopic ones when the dry phase is ending. The differences between the findings presented by Shen et al. (2004) and those by Anderson, Sehested and Ingvartsen (1999) may stem from variations in the length of time taken in giving the nutritional treatments, ration CHO's definite digestibility, and the phase of lactation when manipulations are executed. This presents challenges in predicting the definite morphology-related reactions to changes in the composition of rations (Argov-Argaman, Eshel, Moallem, Lehrer, Uni & Arieli, 2012).

There is ample documentation of the links between ruminal papillae area where absorption happens and fermentability of diet. Even then, there is limited past research relating to expression responses of enzyme along with transporter activity within the walls of lumens (Penner, Steele, Aschenbach and McBride, 2011). Temporal alterations in the functions of cells that constitute epithelial tissues are early reactions to

modifications of diets (Argov-Argaman, Eshel, Moallem, Lehrer, Uni & Arieli, 2012). There is a lack of published studies exploring changes in the structure of the rumen microbial community during the transition period dairy cows. Thus the objective of this study was to study the relationship between the composition of the bacterial community in dairy cows in the rumen during the transition period to identify plans that might improve or avoid NEB through regulation of microbial fermentation in the rumen.

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CHAPTER II. EXPRESSION PROFILES OF RUMEN MICROORGANISMS DURING THE PERIPARTAL PERIOD IN HOLSTEIN COWS

INTRODUCTION

The ruminal microbial populations encompass bacteria, as well as protozoa and fungi. Alterations in diet and intake for dairy cows can impact each group of microbial populations differently (Grant & Albright, 1995). Bacteria have the shortest generation period, and; therefore, alterations in diet and intake have an effect on these populations within the rumen. The protozoa most of the time attach themselves to bigger feed particles or even the ruminal wall to surmount the long generation period. As a consequence, the protozoa usually maintain their populations even during periods of diet and intake. Furthermore, protozoa can utilize bacteria and fungi as a source of nutritional value to sustain their populations in periods of limited intake, as well as low quality diets (Journet & Remond, 1976). On the other hand, fungi attach themselves and feed on fiber particles and; therefore, release fungal spores when they mature. As a result, alterations in diet and intake can significantly impact their populations inside the rumen. The two tiered feeding structure used in this study for Holstein dairy cows was meant to provide time for ruminal microbial populations to fine-tune with dietary changes and; consequently, diminish the incidences of ruminal as well as metabolic ailments especially after calving (Kertz, Reutzel & Thomson, 1991). The ruminal microbes must also react/respond to alterations of intake and diet as the dairy cows make the change from late gestation period into early lactation period. In normal circumstances, ruminal microbial populations in Holstein dairy cows are related with diet and intake alterations and how this changes in different lactation periods. During these periods,

the fungal, protozoa and bacterial populations in the rumen change with regard to dietary and intake changes in far-off, as well as close-up diets. State At the end of the study, it was clear that ruminal microbial populations of Holstein dairy cows usually respond to alterations in diet and intake. Alterations in diet usually affect populations of protozoa as well as fungi. However, a change in intake affects fungi and protozoa populations, as well as bacterial populations (Keown & Grant, 1991; Keys, Pearson & Thompson, 1978).

MATERIALS AND METHODS

Animals and diets

All procedures were performed under protocols approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #12094). Briefly, seven rumen fistulated Holstein cows in their second or greater lactation were selected for this study. Cows were managed according to the University of Illinois standard operating procedures. In addition, cows were fed during the dry period using the two-stage approach with a high-straw, lower-energy diet fed from dry off through -21 d from parturition followed by a lower-straw, and higher-energy diet until parturition (Table 1). Cows were then fed a common lactation diet until day 30 postpartum. Diets were fed as a total mixed ration (TMR) once daily (0600 h) using an individual Calan (American Calan, Northwood, NH, USA) gate feeding system during the dry period or in open individual managers during lactation. Sampling of feed ingredients and TMR for composition analyses, and housing of cows pre and postpartum were as reported previously. Also, details of BW, BCS, milk weights and sampling of milk composition were as described previously (Graugnard et al., 2012; Graugnard et al., 2013). Briefly, cow BW were obtained weekly, and BCS assigned weekly throughout the study. Cows were milked three times daily after parturition and milk was sampled thrice weekly for analysis of chemical composition.

Rumen sampling collection

On days -14, -7, 10, 20, and 28 days around parturition and prior to the morning feeding, a grab sample of ruminal contents was collected via the ruminal cannula from the ventral sac of the rumen after mixing of the contents. The mixed ruminal content was squeezed through three layers of cheesecloth to recover a rumen fluid and rumen solid sample. All samples were

immediately placed on an ice bucket, transported to the laboratory, and stored at -20 °C prior to DNA extraction.

DNA extraction

A 25 g of solids rumen content was used for extraction. The procedure used was that of Stevenson and Weimer (2007) with modifications. To each sample was added 75 mL of chilled Extraction Buffer (EB) which was composed of 100 mM Tris/HCl, 10mM ethylenediaminetetraacetic acid (EDTA), and 0.15 M NaCl at pH of 8.0. The mixture was blended by polytron for 2 min. The probe of the polytron was then rinsed with an additional 25 mL of EB. The resulting mixture was transferred to a 50 mL Falcon tube and centrifuged gently at 500 × g for 15 min at 4 °C to remove the considerable amount of broken plant particles while keeping bacterial cells in suspension. The supernatant was then filtered through a small quantity of glass wool into a new 50 mL Falcon tube.

The resulting supernatant was centrifuged at 10,000 × g for 25 min at 4 C. In addition, and the corresponding 25 mL of rumen fluid from the same cow was centrifuged at 10,000 × g for 25 min at 4 °C. The resulting pellet from the solid and the liquid samples, which contained bacterial cells, were re-suspended in 4 mL ice-cold EB buffer. A volume of 350 µL was aliquoted into a 1.5 mL microfuge tube. Subsequently, to each sample was added 0.25 g zirconium/silica beads (0.1 mm diameter), 25 µL 20% sodium dodecyl sulfate (SDS), and 350 µL equilibrated phenol (pH 8.0). These suspensions were shaken in a bead beater for 2 min, incubated at 60 to 65 °C for 10 min, and then shaken for an additional 2 minutes.

After shaking, the samples were centrifuged at 12,000 × g for 5 min and the aqueous phase transferred to a new 1.5 mL microfuge tube. The aqueous phase was extracted twice with 500 µL

phenol (pH 8.0), twice with 500 µl phenol/chloroform (pH 8.0), and finally twice with 500 µl chloroform at 12,000 × g for 5 min each time. A small amount of EB was added occasionally to keep the aqueous volume above 450 µL. The final supernatant was combined with 0.1 volumes of 3.0 M Na acetate and the bacterial DNA precipitated with 0.6 volumes of isopropanol.

The precipitated DNA pellet was washed twice by adding 1,000 µL of 70% ethanol without disturbing the pellet and centrifuged at 12,000 x g for 5 min. The resulting supernatant was decanted. After a final additional centrifugation, the remaining liquid was removed by aspiration and the DNA pellet dried at room temperature for ~20 min. The pellet was then re-suspend overnight in 50-100 µL Tris-EDTA Buffer (1x TE), which is 10 mM Tris-HCl and 1 mM EDTA at pH 8.0. The extracted DNA was stored at -80 °C until use.

qPCR Analysis

All the samples were diluted DNase/RNase with free water to be obtained an object concentration of 8 ng/µl of DNA. In addition, an equal amount of every sample was used to formulate a pool DNA sample for standard curve. From the pool DNA, a 6 point standard curve was arranged using 1:6 dilution and the obtained consecrations were 60, 10, 1.6667, 0.2778, 0.0463, 0.0077 ng/µl . A total of 4 µl of the diluted sample and standard (8ng/ul) was added to the their corresponding well MicroAmp™ Optical 384-Well Reaction Plate, every sample and standard was run in triplicate. A mix containing of 5 µl 1x SYBR Green and 0.4 µl of 10 µM forward primer with 0.4 µl of 10 µM reverse primer in 0.2 µl DNase/RNase free water was added to each well to achieved final volume of 10 ul for each well. The reactions is completed in an ABI Prism 7900 HT SDS instrument using the following conditions with Quanta (Perfecta) SYBR green: 5 min at 95°C, 40 cycles of 1 sec at 95°C and 30 sec at 60°C. Confirm the specificity of the

amplicons by the dissociation protocol (95°C for 15 s plus 65°C for 15 s); analyze the data using the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems).

Statistical analysis

The MIXED procedure of SAS (SAS Institute, Inc., Cary, NC, USA) was used for statistical analysis of milk production, BW, BCS, milk composition, and percentage of microbes. The fixed effect in the model was time (day or week), and the random effect was cow. The covariate structure used was CS. The method of Denman and McSweeney (2006) was used for quantitative RT-PCR analysis of target microbes. Briefly, the number of cycles (Ct) during PCR for each of the microbes evaluated was first subtracted from the geometric mean of the cycles obtained with the universal microbial primers (ΔCt). The universal primers served as internal reference because their abundance is much greater than specific microbes. Hence, the concentration of the target microbes was calculated relative to the abundance of the universal primers. Prior to statistical analysis, the percentage of each microbe target was calculated as $(2^{-\Delta Ct}) \times 100$. Subsequently, the data were log-2 transformed prior to statistical analysis. All means among time points were compared using the PDIFF statement of SAS (SAS Institute, Inc., Cary, NC, USA). Significant difference was declared at $P < 0.05$.

RESULTS

Rumial bacterial Population during transition period in Holstein cows

Holstein dairy cows experience changes in microbial populations during transition into lactation period. From the study of the seven ruminal bacterial populations during the transition period, the different bacteria experience changes in their populations. In most of the seven bacteria, the population remains low before parturition and increases especially during the early lactation period. However, in the two cases the bacterial population was not significant as shown in the Figure 1A and 1G.

The bacterial population of *Fibrobacter succinogenes* decreases from -7 day prepartum to the tenth day postpartum (Figure 1B). However, immediately after 10^{th} day of parturition, the bacterial population increases significantly. Similarlar pattern was observed for *Anaerovibrio lipolytic* bacteria whose population rate remains low before parturition, that is, between -14 to -7 days (Figure 1C). Nevertheless after parturition, the population for these bacteria increased during the early periods of lactation and eventually started decreasing from 20 days onward. However, this is not the case for *Megasphaera elsdenil* bacteria since, the bacterial population remains high both before and after parturition (Figure 1D). However, after the early days of lactation, the bacteria experiences slight decreases in their population. In addition, the bacterial populations remain stable prepartum.

The population of *Prevotella bryantii* bacteria remains high the during the entire period, both before and after parturition (Figure 1E). Conversely, for the *Butyrivibrio Proteoclasticus* bacteria, the bacterial population decrease continuously during the entire study period. In general, during the late gestation period, most of the ruminal bacterial populations remain low even during far-off and close-up diet periods. However, during early lactation period, the ruminal bacterial

population increases significantly. The increase in bacterial populations is mainly due to the change in lactation diet after calving. In addition, the observed decrease in ruminal bacterial population decrease is as a consequence of an increased starch intake. This reduces the ruminal bacterial population.

Alteration in milk productivity in response to change ruminal bacterial population

The table (4) depicts the changes and the relative body weight (BW) alterations after delivery and their connection to milk yield in Holstein cows. The table also depicts the relationship between the alterations in BW and reproductive performance in the first 4 weeks of lactation and the last 3 weeks before parturition. From the table above, it is clear that there is a relative decrease in the body weight of dairy cows after delivery. In addition, the amount of milk before delivery reduces and increases towards the last weeks before delivery. After parturition, the milk yield increases considerably up to the fourth week. The Holstein cows particularly produce more milk two weeks after delivery as indicated in the table above.

The milk composition also depicts significant changes in the phase after parturition. After delivery, the percentage of fat in milk reduces considerably from the first week of lactation up to the fourth week. In addition, the percentage of protein also reduces not too much in the first two weeks of lactation, but has a significant decline in the third and fourth week. There is also a slight decline between the first and fourth week of lactation in the percentage of lactose as well as the MUN. The somatic cell count (SCC) also records a decline. However, there are no major differences in the second and third weeks of lactation. From the graph above, the daily milk production in prepartal Holstein cows fluctuates significantly. The fluctuation is not too much fifteen days prior parturition. However, the fluctuation increases at least fifteen days to delivery.

The milk yield increases in preparation of the calving period, as well as the lactation phase. These can also due to changes in diet.

DISCUSSION

Bacteria:

In ruminant animals, the ruminal bacterial population is extremely responsive to changes in diet, intake, age, feeding structure, as well as the condition of the host dairy animal. Prepartal diet usually has the capacity to ease enabling the rumen microbes as well as papillae to the high-starch diets administered during lactation period. The transition phase for dairy cows which is usually three weeks before and after calving poses great challenges to dairy cows. This is because, the nutritional requirements change significantly during this period and in most cases the nutritional requirement increases during this phase. This is usually to maintain the fetal development, as well as the onset of milk production (Bell, 1995). Nonetheless, DMI mostly diminishes one week prior to parturition and eventually increases to three weeks after birth. The ruminal microbial populations and ecosystem is a crucial aspect that connects diets to ruminant physiology, as well as productivity. There are constant changes in the ruminal microbial populations with regard to dietary alterations in dairy cows (Bergman, 1990). A noteworthy increase and change in diet and intake has a resultant increase in the population of Eubacterium ruminantium. However, increasing the intake of dry matter in dairy cows results in a reduced Eubacterium ruminantium bacterial population (Bertics et.al, 1992). However, Fibrobacter succinogenes as well as fungal populations usually increase during the post feeding phase. In addition, during the transition period, the population numbers of major propionate-producing micro-organisms, that is, Megasphaera elsdenii as well as Selenomonas ruminantium reduces (Bhandari et.al, 2008).

During this period, glucose levels as well as volatile fatty acid (VFA) diminish while the lactic acid levels increase in the transition period. In the transition period, dairy cows undergo a

dramatic change from pregnancy to the delivery period, as well as the lactation period. These dramatic changes are mainly metabolic changes in the dairy cattle which might bring about health disorders for instance ketosis. As a result, the transition phase is a crucial stage to the health, production as well as the profitability of dairy cattle. These changes call for the need to fulfill the nutritional needs of high-producing cows especially during the calving period. After parturition, six weeks is defined as the period for udder tissue regeneration, as well as rumen wall recreation. Five weeks after parturition the cow is supposed to maintain stable weight or the cow might contract a metabolic disorder (Bryant, 1959). It is crucial to prepare properly before and after parturition so as to ensure that the dairy cows attain the best chance to maintain their highest genetic potential. As a result, post-calving nutritional needs determine what happens. Adjusting the rumen ecosystem, that is, bacterial populations inside the rumen after giving birth is crucial. During this period, the diet supplied to the cow is mainly used for repairing the rumen, as well as the udder tissue. During this phase of restoration and regeneration of the udder tissue and rumen, ruminal bacteria populations decrease. The bacteria populations also change in their type quantities. These populations usually change with regard to high fiber, limited energy, as well as protein diet (Caldwell, 1966). The rumen papillae, that is, the absorption sites also reduce in size and capability. This explains the results above, and the different fluctuations in ruminal bacterial populations due to this regeneration period. It is crucial therefore to introduce feeds to foster feed-specific ruminal bacteria populations which can increase in numbers after parturition. The bacterial multiplication in the rumen is crucial in reducing chances of rumen upset, for instance, acidosis (Chandler, 1995). This is because introducing new feeds (grain or pasture) helps in increasing rumen papillae and; hence, enhancing the nutrient uptake capacity. It is crucial to note that most of the dairy cow's nutrients absorption is usually as a result of microbial

fermentation, as well as modification. This is also dependent on what the cow consumes be it pasture or grain. As a consequence, the ruminal microbial populations exist in an extremely dynamic environment/condition and significant alterations usually occur with changes in diet. The crucial change in the ruminal bacteria population and fermentation procedures occurs in the rumen and; therefore, the fermentation becomes considerably dynamic. In addition, there is a further decreased ruminal retention period of feed, as well as ruminal bacteria. Fermentation within the rumen translates into increased VFA production and a reduced pH. The rumen is usually buffered at a pH of 6.8 and a pH below 6.0 translates into a bad situation. This is because below a pH below 6 reduces the breakdown of fiber (Coe, 1997). As a result, the capability and the time required for the ruminal bacteria populations to adjust to these alterations are consequently extremely critical for the dairy cows (Cole, 2007).

The transition period is extremely critical in the life of dairy cattle. Three weeks prior calving and three weeks after parturition, the dairy cow undergoes varied hormonal, metabolic as well as dietary alterations in preparation for delivery as well as the impending lactation. This is a critical phase for any dairy cow productivity, as well as health. Therefore, it is crucial to adequately prepare for this phase so as to maintain the genetic potential of dairy cattle. Maintaining this potential comes in handy in ensuring proper milk production and sustaining the health of the cattle. This is because, when the rumen does function properly, feed digestion is reduced and the dairy cattle also becomes more vulnerable to a variety of metabolic ailments, milk production also goes down drastically. As a result, rumen adaptation engages the ruminal microbial populations as well as rumen wall and other supporting organs and functions. The dairy cow usually has reduced diet before delivery. However, immediately after calving the diet is usually energy dense with libitum to supply the cow's energy as well as nutritional needs

during the lactation process. The increased dry matter intake therefore reduces rumen fermentation and; hence the reduced ruminal bacteria populations. Nonetheless, it is important to note that the transition phase is related with a reduction in feed intake. During this period, the energy, as well as nutritional needs is usually double. The slow reductions in the dairy cattle' voluntary DMI starts at 3 weeks before delivery and increases during the final days prior to delivery. This is critical in countering the negative energy balance, the deficiency in glucose and proteins and to quickly supply the required energy, as well as productivity. Another issue with regard to the rumen's changes is that, the rumen must effectively adjust to fulfill the cow's energy and nutritional needs. In the case that the diet of a dairy cow is changed abruptly from high forage feed to a increased concentrate diet, then, there is a possibly that the cattle might develop ruminal acidosis since the lactate producers increase much more quickly as compared to the lactate users. As a consequence, lactate accumulates and the rumen pH reduces significantly. When the rumen absorption sites are not properly developed, the VFA are not adequately absorbed and there is a consequent drop in rumen pH, the killing of majority of the ruminal bacteria and protozoa. This is referred to as sub-acute ruminal acidosis (SARA) which is a metabolic disorder related with a reduction in ruminal pH, as well as reduced VFA intake. There is also endotoxin production and this can result in decreases in feed intake and; consequently, enhance the unconstructive energy balance of the dairy cow in transition. In making sure that the rumen performs properly and its microbial populations and rumen wall adjust well to impending lactation phase, it is critical for the cow to be given a diet that is easily and quickly fermentable. This is mostly roughly two weeks prior to delivery. In addition, efforts should be made to enhance the voluntary absorption to prevent any metabolic disorders and related conditions that may reduce milk production. This must be in consideration of the inherent feeding regime and

the components, as well as the feed's degradation properties. The microbial procedures and the intra-ruminal aspects also come in handy including the rumen wall and the cow's regulations with regard to saliva production, intake of VFA and other nutrients. In addition, the feeding times and regularity is also crucial in influencing the ruminal microbial populations (Counette et.al, 1981).

The rumen epithelium response capacity is also crucial during the transition period. The epithelium plays a crucial role in buffering the rumen during early lactation phase. Rumen adaptation to the dietary changes especially the lactation diet involves alterations in enzyme activities and an increased quantity as well as size of the absorption roots of the rumen as well as the transportation speeds of the ions. The rumen absorption roots development during the transition phase is very much related to a good VFA absorption. This is because the rumen papillae size and shape enhances the surface area so as to enhance the absorption of the VFA. It is however crucial to note that dairy cattle have a high capability to adjust the rumen wall to the intrinsic-ruminal fermentation aspects (Denmann, 2006). Nonetheless, early functional adjustment outside the rumen epithelium acts as the initial stage in tackling changed fermentation rates after dietary changes. Functional adaptation usually occurs within a week into novel diet while structural adjustments occur roughly six weeks after parturition. The heightened energy as well as protein absorption of the dairy cow quickly leads to increased rate for transport of crucial body minerals such as sodium, magnesium, as well as calcium. There is also heightened VFA absorption, as well as bicarbonate secretion. The transition adaptation phase has not more than ten days to bring about increased VFA concentrations in the rumen as a result f the epithelium's inadequate buffering capacity (Fernando, 2010).

Prepartum feed is usually different from the feed supplied after parturition. The traditional mechanism of feeding dairy cattle with high forage feed which are high in fiber is common in the prepartum phase. However, it is crucial to note that the lactation diet influences the bacterial population inside the rumen, the size as well as the absorptive capacity of the rumen absorption sites. Nonetheless, there is need to control the amount of feed the cattle take since it is possible for the cattle to consume more energy than necessary. Higher fiber feeds which are low in energy can foster DMI after delivery and reduce extreme complications in the adipose tissue. This type of feed helps reduce cases of displaced abomasum, enhanced body condition, reproductive capacity, as well as enhancing the cow's foot health. As delineated above, ruminal microbial populations of dairy cattle usually respond to alterations in diet and intake during the lactation period. These changes in diet also influence the protozoa and fungi populations in the rumen of dairy cattle. These changes can be properly dealt with, with adequate preparation in the prepartal period, as well as after delivery. This preparation is extremely crucial in dealing with dietary adjustments. Different diets can be introduced early before and after parturition to allow for the animal's system to prepare for any changes with regard to delivery and lactation. This is because these two phases have different energy requirements which must be provided in order to ensure continued milk production and good health of the dairy cow. In a case where these dietary requirements for the dairy cow are not met, then, there is the possibility of health, biological and metabolic disorders. It is also critical to note that, the factors influencing the changes in ruminal bacteria populations are dependent on a variety of factors. These factors include the type of feed provided to the dairy cow, the frequency and time of feeding, the age of the cattle, the period before and after delivery, as well as the management of the cow's dietary needs. The above discussion reinforces on the fact that, the increase in bacterial populations is mainly due to the

change in lactation diet after calving. On the other hand, the observed decrease in ruminal bacterial population is as a consequence of an increased starch intake which reduces the ruminal bacterial populations (Goad, 1998; Gottschalk, 1986; Grummer, 1995; Henning et.al, 2010; Hino et.al, 1994; Hungate, 1996; Kent et.al, 2003; Khafipor et.al, 2003; Marounek et.al, 1989; 1987; 1989; Moe, 1972; Nisbet, 1991; Reynolds et.al, 1988; Shyu, 2007; Stewart et.al, 1988; Tajima, 2001; Varga, 2001; Xu, 2010; Denman, 2006).

CONCLUSIONS

The transition phase is a very crucial period in the life a dairy cow. It is a phase accompanied by many changes including health, biological, physical, as well as metabolic changes. It is crucial to ensure that dairy cattle adapt to the metabolic changes that occur during this phase so as to reduce their vulnerability to infectious ailments and metabolic disorders. These changes also have a significant influence on the ruminal bacteria populations which must be taken into consideration given the importance of the ruminal bacteria in the digestion processes of dairy cattle. This is because soon after calving, there is repair of the rumen, as well as the udder tissue. As a result, ruminal bacteria population reduces due to the high fiber, low energy and protein diet. The rumen absorption sites also reduce in size and capability. This is because more energy and nutrients are utilized in the regeneration of tissues than in the fermentation process related with feed breakdown. The dietary changes for the dairy cattle are the major aspects behind the alterations in ruminal bacteria populations inside the rumen. As a result, the feeding strategies and preparation are crucial in fostering better management of dairy cattle and in preventing the complications that come with the transition period. Comprehending the aspects that affect the composition of the ruminal bacterial populations of the digestive tracts

of dairy cattle is also crucial in controlling these populations to foster and enhance animal performance and productivity.

Milk:

There are a lot of changes in the milk yield in Holstein cows especially during the transition period. This is attributed to the intake and dietary changes that accompany this phase. During the transition period, that is late gestation and early lactation period, dairy cattle transition from feeds that are high in forage but low in energy to feeds that are high in energy but low in forage. This affects the body weight and the milk production of the dairy cattle. The milk yield of dairy cattle relies on a variety of factors. These factors include genetic ability, the feeding regime, herd management, as well as the health of the dairy cattle. As the dairy cattle continue to advance genetically, the nutrition as well as the management of the cattle must also be improved to allow the cattle to produce adequately to their genetic potential. A good feeding regime considers the quantity of feed, the appropriateness of the feed and the frequency and of the feeding. During the lactation period, dairy cattle are fed on DMI. Intake of huge amounts of DMI is crucial in increasing milk production and fostering effective milk production. Proper selection of feeds is crucial in ensuring maximum nutrient intake. This is because all the necessary nutrients for dairy cattle for production of milk are present in the DMI except water. As a result, the DMI which is usually administered after parturition leads into increased nutrient intake, as well as high milk yield. This is as recorded in the milk table, where is a recorded increase in milk yield after parturition. It is also crucial to note from the graph that the milk production slightly increases during the late gestation period up to the early lactation period. However, maximum DMI must be complemented with constant access to clean, fresh, as well as cool waters. The milk production levels usually rise at approximately three weeks after delivery.

However, milk production decreases at around one year after calving until the milk production ceases to the dry off period.

Milk components are usually an indicator of a dairy cow's heath, as well as nutrition. In many cases, fat and protein component values in milk are positively linked inside a population of dairy cows. Nonetheless, it is crucial to note that, divergent breeds of dairy cows have different milk components. Holstein dairy cattle usually have the lowest fat, as well as protein content as depicted in the milk table above. However, these cattle produce a lot of milk and; therefore, they have a higher total production of fat and protein compared to other breeds such as Jersey. There are varied factors that affect the milk composition of Holstein dairy cattle. The declining milk composition as presented above could be linked to aspects to do with nutrition, feeding regime, rumen health, as well as body condition. Other factors might include the management of feeds, energy and protein impacts, as well as concentrate intake and forage levels in the diet. However, of all these factors, nutrition and the feeding regime have the greatest impact. In addition, the management structure can quickly and drastically change the production of milk protein, as well as fat. A reduction in milk fat usually reduces between seven and twenty ones of altering the dairy cattle diet. The changes in milk protein usually occur if this situation has been going on for a long while. Additionally, nutrition, as well as ration formulation alteration is very strongly linked to milk fat value than milk protein. Consequently, the decline in milk protein and fat component and fat component can be linked to the nutrition and the feeding regime of the Holstein cattle.

The milk components emanate from digestion of fiber within the rumen. The rumen ruminal microbe populations usually convert protein in the cows' diet into microbial protein which is a crucial source of essential amino acids for the dairy cattle. The resultant amino acids

are utilized by the mammary glands to breakdown milk proteins. Therefore, the supply and amount of amino acids influence the amount of protein components available in the cow milk. In addition, the feeding management affects the production of milk due to the divergent dietary requirements of dairy cattle in the prepartal and post-partal periods. If the right dietary needs and intake are not provided especially before and after parturition, then, the milk yield reduces significantly. The amount and type of feed provide for lactating animals should be adequate for the ongoing body repairs (rumen regeneration and repair of the udder tissue), as well as the cow's daily dietary needs. The nutrients in the feed should be adequate in supplying the cow with energy and at the same time, producing enough milk for the calf. A poor feeding regime and management often results in a decrease in milk yields. The health condition of a cow also influences milk production. Good health condition is crucial in allowing dairy cows to produce enough milk, and ensuring that the body also stores sufficient nutrients to foster milk yields. This is because, if the cow's body stores are limited, milk production and the milk components are also affected. However, disproportionate body condition may result in high chances of metabolic quandaries. The loss of cattle weight during early lactation period can also play a critical role in increasing protein component in milk.

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Table 1. Feed analysis composition of dry matter for the total mixed ration in each pried.

	TMR (-14)[1]	TMR (-7)	TMR (+10)	TMR (+20)	TMR (+28)
	DM[2]	DM	DM	DM	DM
% Crude Protein	15.175	14.96	17.38	17.557	17.325
% Adjusted Crude Protein	15.175	14.96	17.38	17.557	17.325
% Acid Detergent Fiber	22.975	23.94	22.78	23.471	23.425
% Neutral Detergent Fiber	33.6	34.52	31.9	34.243	35.45
% TDN[3]	71	70.8	71.6	70.857	70.5
NEL, Mcal/Lb[4]	0.758	0.754	0.766	0.757	0.753
NEM, Mcal/Lb[5]	0.765	0.76	0.772	0.759	0.753
NEG, Mcal/Lb[6]	0.49	0.484	0.494	0.481	0.475
% Calcium	0.98	0.994	1.166	1.124	1.105
% Phosphorus	0.328	0.32	0.418	0.42	0.408
% Magnesium	0.45	0.444	0.334	0.319	0.313
% Potassium	1.215	1.222	1.058	1.059	1.038
% Sodium	0.059	0.061	0.278	0.279	0.27
PPM[7] **Iron**	457.5	448.8	498.8	469.714	477.25
PPM Zinc	295	256.4	244.8	202.714	185
PPM Copper	55	48	40.6	33.857	31.75
PPM Manganese	305.25	264.2	211.2	172.714	162
PPM Molybdenum	0.825	0.78	0.94	0.943	0.925
Horse DE[8]**, Mcal/Lb**	1.253	1.24	1.262	1.234	1.223

[1]Total Mixed Ration.
[2]Dry Matter.
[3]Total Digestible Nutrients.
[4]Net Energy-Lactation.
[5]Net Energy-Maintenance.
[6]Net Energy-Gain.
[7]Parts Per Million.
[8]Digestible Energy.

Table 2. Ingredient composition of diets fed during closeup (−14 days to calving), and early lactation (after calving to +28 days).

Ingredient (% of DM)	%Diet %DMI Close-up	%Diet %DMI Lactation
Alfalfa silage	7.815	11.34
Alfalfa hay	6.685	7.78
Corn silage	36.685	18.94
Wheat straw	11.075	3.74
Cottonseed	-	4.84
Wet brewers grains	1.57	10.145
Dry Ground corn grain	17.06	22.345
Soy hulls	6.44	5.21
Dried Corn gluten feed	1.795	6.375
CU mix	10.865	-
Lactation mix	-	9.29

Table 3. Organism, hybridization position, and sequence of primers used to analyze the composition of rumen microbes by qPCR.

Organism	Primers	Primers (5'-3')	Source
Eubacterium ruminantium	F.	CTCCCGAGACTGAGGAAGCTTG	Stevenson and Weimer (2007)
	R.	GTCCATCTCACACCACCGGA	
Butyrivibrio proteoclasticus	F.623	GGGCTTGCTTTGGAAACTGTT	This study
	R.723	CCCACCGATGTTCCTCCTAA	
Anaerovibrio lipolytica	F.92	GAAATGGATTCTAGTGGCAAACG	This study
	R.211	ACATCGGTCATGCGACCAA	
Fibrobacter succinogenes	F.	GCGGGTAGCAAACAGGATTAGA	Stevenson and Weimer (2007)
	R.	CCCCCGGACACCCAGTAT	
Megasphaera elsdenii	F.	AGATGGGGACAACAGCTGGA	Stevenson and Weimer (2007)
	R.	CGAAAGCTCCGAAGAGCCT	
Prevotella bryantii	F.	AGCGCAGGCCGTTTGG	Stevenson and Weimer (2007)
	R.	GCTTCCTGTGCACTCAAGTCTGAC	
Selenomonas ruminantium	F.	CAATAAGCATTCCGCCTGGG	Stevenson and Weimer (2007)
	R.	TTCACTCAATGTCAAGCCCTGG	

Table 4. Sequencing results of PCR products from primers of microbes utilized for this experiment. Best hits using BLASTN (http://www.ncbi.nlm.nih.gov) are shown.

Organism	Sequence	Description
Eubacterium Ruminantium	CAGTACTTTGAGTACTTAGTCAGGCAGGAC GGTGAGTACGCGTGTGATACCTGCCTCACA CAGGGGATAACATCGTTAGGAACGACCTGT TAATACCGCATAAGCGCACAGTATCGCATG GTACAGTGTGAAAAACTCCGGTGGTGTGAG ATGGACAAGCTTCCTCAGTCTCGGGAGA	Eubacterium ruminantium strain GA195 16S ribosomal RNA
Butyrivibrio proteoclasticus	CGATCTGGAGTAGCGATCCTAGTGTAGCGG TGAATGCGTAGATATTAGGAGGAACATCGG GTGGGACC	Butyrivibrio proteoclasticus B316 strain B316T 16S ribosomal RNA gene
Anaerovibrio lipolytica	GGTACTGCGATGCACTGACCTTCAGATGGG GACACATTTCGAAAGGAAGTGCTAATACCG AATGACGTGCATTGGTCGCATGACCGATGT CAGC	Anaerovibrio lipolytica DSM 3074(T), 16S rRNA gene
Fibrobacter succinogenes	CCAGTGATCTCGCAGCNTACGAGTCATACT GGGTGTCCGGGGGA	Fibrobacter succinogenes subsp. succinogenes S85 16S ribosomal RNA gene
Megasphaera elsdenii	CGACTACTCGAAATCGTTCTTTGTCGCATGG CAGAGAGAGAAGGGAGGCTCTTCGGAGCTT TCGAATCTA	Megasphaera elsdenii strain YJ-4 16S ribosomal RNA gene
Prevotella bryantii	GCGTTAGTCGGGCCTACTGGCACTGCAGCG CGANCTGTCAGACTTGAGTGCACATGGAAG CANCGTTAAAACGC	Prevotella bryantii strain 3C5 16S ribosomal RNA gene
Selenomonas ruminantium	CGATCGAGGCAGTACTCAAGGATTGACGGG GGCCCGCACAAGCGGTGGAGTATGTGGTTT AATTCGACGCAACGCGTAAGAACCTTACCA GGGCTTGACATTGAGTGAAA	Selenomonas ruminantium strain 3F11L 16S ribosomal RNA gene

Table 5. Body weight, milk yield, body condition score, and milk composition in peripartal Holstein dairy cows.

	Week relative to parturition									
Item	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**SEM**	***P* =**
Body weight (kg)	807[a]	806[a]	799[a]	736[b]	700[bc]	669[c]	630[c]	632[c]	26	0
BCS[1]	3.77[a]	3.75[a]	3.85[a]	3.68[ab]	3.61[b]	3.32[c]	3.14[cd]	3.00[d]	0.13	0
Milk, kg	--	--	--	--	29.2[b]	36.3[ab]	38.2[a]	41.1[a]	4.2	0
Milk composition										
Fat (%)	--	--	--	--	5.85[a]	4.95[b]	4.23[c]	4.30[c]	0.36	0
Protein (%)	--	--	--	--	3.89[a]	3.24[b]	2.89[c]	2.78[c]	0.08	0
Lactose (%)	--	--	--	--	4.29[b]	4.56[a]	4.66[a]	4.62[a]	0.07	0
MUN (mg/dL)[2]	--	--	--	--	14.7[a]	14.1[a]	12.2[b]	12.8[b]	0.83	0
SCC (log10)[3]	--	--	--	--	1.93	1.62	1.67	1.88	0.18	0.21

[a-c]Different superscripts denote differences between weeks ($P < 0.05$).

[1]Body condition score.

[2]Milk urea N.

[3]Somatic cell count.

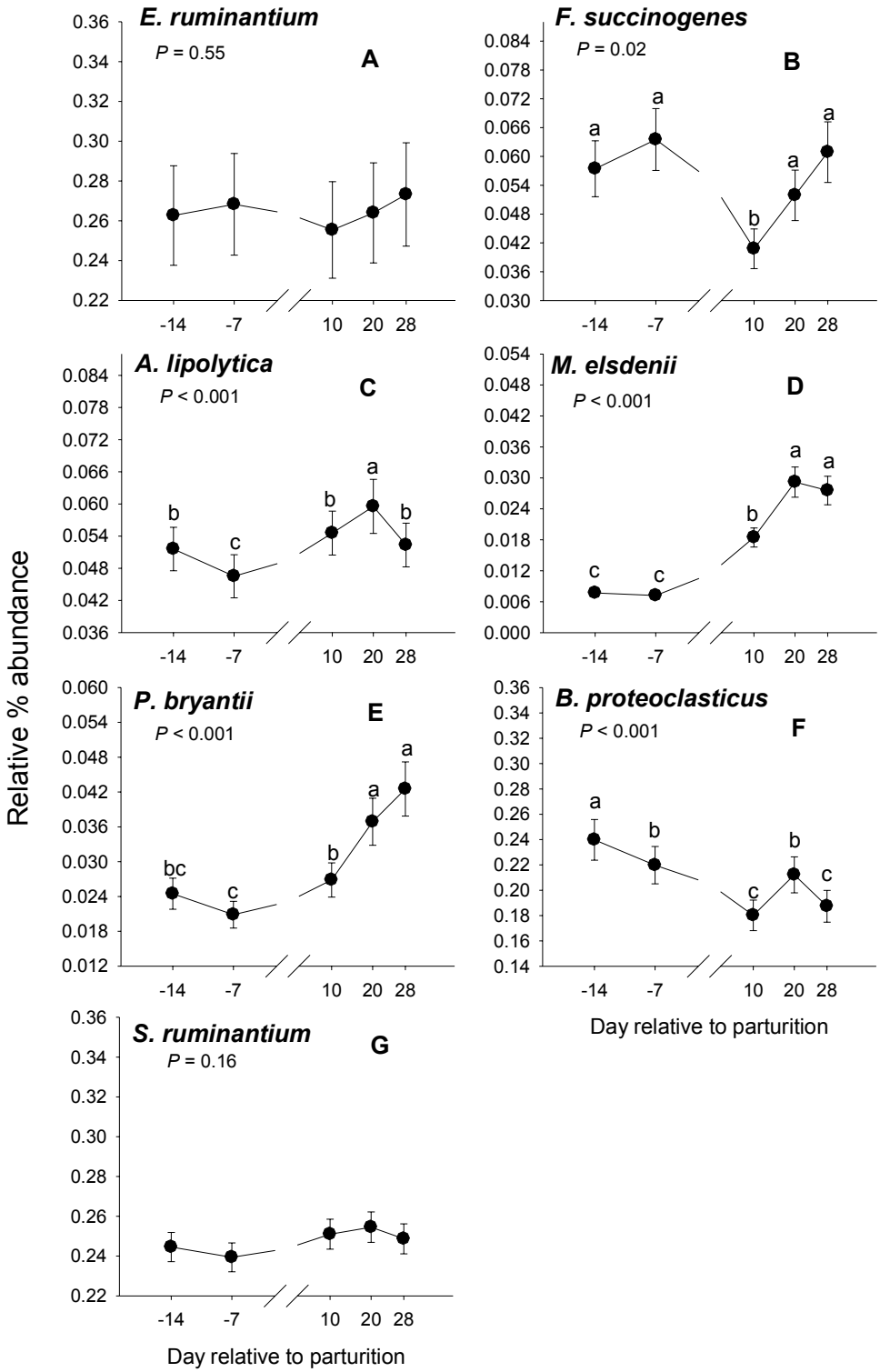

Figure 1. Expression profile of seven ruminal microorganisms during the peripartal period. The y-axis denotes the bacterial species relative to universal percent abundance and x-axis denotes the days relative to parturition. Different letters (a-c) indicate differences due to the main effect of time (P< 0.001).

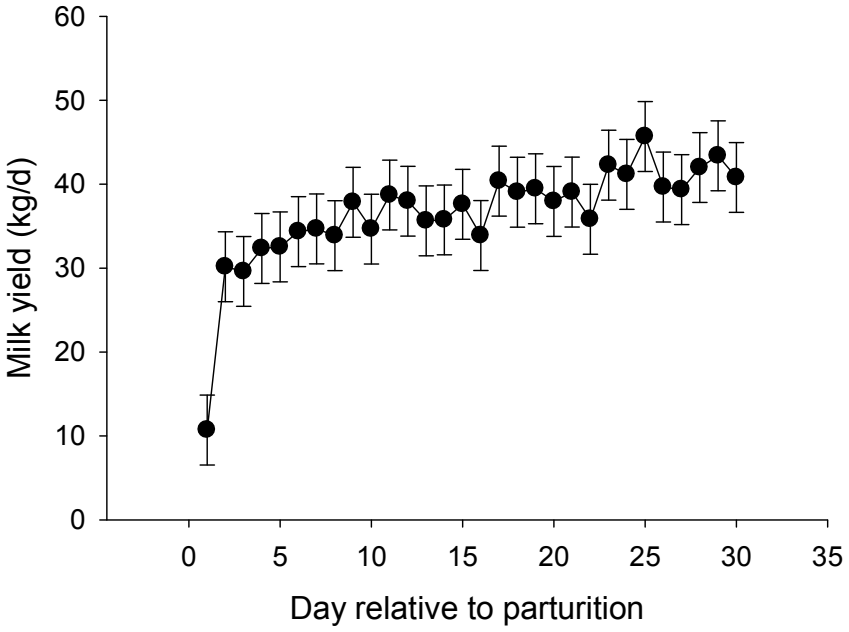

Figure 2. Daily milk yield in peripartal Holstein dairy cows. There was a significant effect of time ($P < 0.05$) on milk yield.